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Integron involvement in environmental spread of antibiotic resistance

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The spread of antibiotic-resistant bacteria is a growing problem and a public health issue. In recent decades, various genetic mechanisms involved in the spread of resistance genes among bacteria have been identified. Integrons – genetic elements that acquire, exchange, and express genes embedded within gene cassettes (GC) – are one of these mechanisms. Integrons are widely distributed, especially in Gram-negative bacteria; they are carried by mobile genetic elements, plasmids, and transposons, which promote their spread within bacterial communities. Initially studied mainly in the clinical setting for their involvement in antibiotic resistance, their role in the environment is now an increasing focus of attention. The aim of this review is to provide an in-depth analysis of recent studies of antibiotic-resistance integrons in the environment, highlighting their potential involvement in antibiotic-resistance outside the clinical context. We will focus particularly on the impact of human activities (agriculture, industries, wastewater treatment, etc.).

Keywords: integron, antibiotic resistance, soil, aquatic ecosystems, wastewater, agriculture, water

Bacterial evolution has largely been shaped by the high plasticity of bacterial genomes, leading to their adaptation to most ecosystems. This ability to exchange and rearrange genomic sequences to gain new traits has been extensively demonstrated with the bacterial resistance to antibiotics. Today, the increasing rate of antibiotic resistant bacteria is a major public health issue (Davies and Davies, 2010). During the last decade, several studies have underlined the environmental resistome as a source of resistance genes of clinical interest (D'Costa et al., 2006; Aminov and Mackie, 2007; Martínez, 2008; Wright, 2010). While mutation events contribute to the bacterial adaptation, horizontal gene transfer seems to be the main cause of the rapid proliferation of antibiotic-resistance genes across a wide diversity of bacteria. Much of this horizontal gene transfers have been shown to occur in the environment (Aminov, 2011). Nevertheless, the diversity of mobile genetic elements currently described (Wozniak and Waldor, 2010; Bertels and Rainey, 2011), shows that beyond horizontal gene transfer, the loss and acquisition of functional modules are an important part in the processes of rapid bacterial adaptation and development of resistance. Integrons are one of the genetic elements involved in the adaptation of bacteria. We address the question of the involvement of integrons in the environmental spread of antibiotic resistance. More specifically, the anthropogenic impacts, which have been shown to be involved in the antibiotic-resistance spread in the environment, and the role of integrons in this process.

INTEGRONS: GENERALITIES

Integrons are bacterial genetic elements able to promote acquisition and expression of genes embedded within gene cassettes (GCs; Stokes and Hall, 1989). The definition of an integron is based on a functional platform (also called 5′ conserved segment, 5′CS), composed of three key elements: the *intI* gene, a specific recombination site *attI*, and a promoter, Pc (Hall and Collis, 1995; Boucher et al., 2007; **Figure 1**). The *intI* gene encodes an integrase protein IntI, which belongs to the family of tyrosine recombinases (Nunes-Düby et al., 1998).

The GCs are non-replicative mobile elements, which generally couple an open reading frame (ORF) with an *attC* site. GCs are integrated or excised from the functional platform by a site-specific recombination mechanism catalyzed by the IntI integrase. Two types of recombination can occur (**Figure 1**): (i) between *attI* and *attC* sites, resulting in the insertion of GCs at the *attI* site, and (ii) between two *attC* sites, leading to excision of the GCs (Mazel, 2006). The GCs can be found either as a linear form, included in an integron, or as a covalently closed circular free intermediate (Collis and Hall, 1992). GCs are usually promoterless and require the Pc promoter for their expression as in an operon. The consequence of this system is that the last integrated cassette is the closest to the Pc promoter (Collis et al., 1993; Collis and Hall, 2004), leading to the highest level of expression in the integron.

Two major groups of integrons have been described: "chromosomal integrons" (CIs), and "mobile integrons" (MIs). CIs are

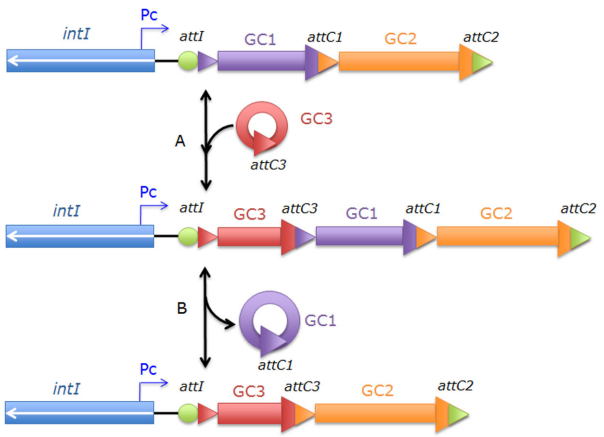

FIGURE 1 | General organization of an integron and gene cassette (GC) recombination mechanism. The IntI1 protein catalyzes the insertion **(A)** and excision **(B)** of the GC in the integron, with GC integration occurring at the *attI* recombination site. In example **(A)**, the circularized GC3 is integrated in linear form inside the integron platform *via* a specific recombination mechanism between the *attI* site and the *attC3* site of the GC3. GC excision preferentially occurs between two *attC* sites. In example **(B)**, the GC1 is excised following the recombination between the two *attC1* and *attC3* sites. Pc: gene cassette promoter; *attI*: integron recombination site; *attC1*, *attC2*, and *attC3*: *attC* GC recombination sites; *intI*: the integrase gene; GC1, GC2, GC3 are the gene cassettes, and arrows indicate the direction of coding sequences (Barraud and Ploy, 2011).

located on the chromosome of hundreds of bacterial species; *in silico* analysis showed that 17% of sequenced bacterial genomes exhibited such genetic arrangements (Cambray et al., 2010). CIs are often described in bacteria from marine or terrestrial ecosystems, such as *Vibrio* spp. and *Xanthomonas* spp., CIs have also been termed "super-integrons" (SIs) as they can carry up to 200 cassettes that mainly encode proteins with unknown functions. CIs may also carry cassettes without functional reading frames. MIs are not self-transposable elements but are located on mobile genetic elements such as transposons and plasmids, which promote their dissemination among bacteria. MIs contain a limited number of GCs (less than 10 GCs; Naas et al., 2001; GenBank DQ112222). The GCs described to date in these MIs usually encode antibiotic-resistance determinants. MIs are therefore sometimes also called "resistant integrons" (RIs) or "multidrug resistance integrons" (MRIs).

In this review, we will focus on MIs.

CLASSES OF MIs

Most MIs have been described in a wide range of Gram-negative bacteria, and only sporadically in Gram-positive bacteria (Martin et al., 1990; Nesvera et al., 1998; Nandi et al., 2004; Shi et al., 2006; Xu et al., 2010; Barraud et al., 2011). Based on the amino-acid sequence of the IntI protein, five classes of MIs have been described (Cambray et al., 2010). Classes 1, 2, and 3 are the most commonly detected. Classes 4 and 5 have only been detected once (Hochhut et al., 2001; GenBank AJ277063).

Class 1 MIs have been extensively studied due to their broad distribution among Gram-negative bacteria of clinical interest and are the most reported in human and animals. They have been described to be mainly associated with functional and non-functional transposons derived from *Tn*402. The non-functional type is the main common structural organization described in clinical isolates, and led some authors to call these class 1 MIs, "clinical integrons" (Gillings et al., 2008c). In addition, these structures are frequently embedded in plasmids or larger transposons, such as those of the *Tn*3 family (*Tn*21, Tn1696) allowing their dispersion (Labbate et al., 2009). The *intI1* gene sequence is highly conserved among MIs found in clinical isolates, while it shows variability in MIs-containing environmental isolates (Gillings et al., 2008b). Furthermore, many class 1 MIs exhibit a 3′ region usually called 3′-conserved segment (3′CS). However, some authors consider using this 3′CS to detect MIs could create a bias in detection, since some MIs lack this sequence (Betteridge et al., 2011). The 3′CS is composed of a *qacEΔ1* gene, a functional deletion of the *qacE* gene still conferring resistance to quaternary ammonium compounds (QACs; Paulsen et al., 1993), followed by a *sul1* gene conferring resistance to sulfonamides, and *orf5* encoding a protein of unknown function.

Class 2 MIs are the second most described group. In most class 2 MIs, the *intI2* gene is interrupted by a stop codon, resulting in a truncated and non-functional protein. This results in a stable GCs array, mainly composed of the GC *dfrA1* (involved in the resistance to trimethoprim), *sat2* (involved in the resistance to streptothricin), *aadA1* (involved in the resistance to streptomycin and spectinomycin), and *orfX* (unknown function; Hansson et al., 2002). However, some class 2 MIs with a different GCs array have been described, probably resulting from the ability of the integrase of class 1 MIs to catalyze recombination at the *attI2* site (Biskri and Mazel, 2003; Ahmed et al., 2005; Ramírez et al., 2005, 2010; Gassama Sow et al., 2010). Class 2 MIs are almost always associated with the *Tn*7 transposon and their derivatives, hence promoting their dissemination. Two class 2 MIs have been described recently with a functional integrase, one containing nine GCs encoding unknown function and the second one harbored the *dfrA14* GC followed by a second novel GC in which a lipoprotein signal peptidase gene has been predicted (Barlow and Gobius, 2006; Márquez et al., 2008).

Only few class 3 MIs have been described. Although their role in clinical antimicrobial resistance is less important, environmental ecosystems could harbor an important pool of these elements (see below).

MIs DISSEMINATION AND THEIR INVOLVEMENT IN ANTIBIOTIC RESISTANCE

Antibiotic pressure has probably played an important role in the MIs selection and dissemination in bacteria. More than 130 GCs conferring resistance to antibiotics and more than 60 GCs of unknown functions have been described in MIs (Partridge et al., 2009). Genes involved in resistance to almost all antibiotic families are embedded in GCs, including beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, fosfomycin, macrolides, lincosamides, rifampicin, and quinolones. In addition, the *qac* GCs, encoding resistance determinants to antiseptics of the QACs family, are commonly found in MIs. Studies have suggested that MIs were more prevalent in bacterial communities subjected to direct or indirect antibiotic pressure in clinical, agricultural, and

environmental settings (Skurnik et al., 2005; Daikos et al., 2007; Barlow et al., 2009; Luo et al., 2010; Kristiansson et al., 2011). Other factors, such as QACs or heavy metals have also been shown to be involved in the MIs dissemination, and thus probably playing a role in their spread before the antibiotic era (see below). More generally, it has been shown, when studying animal fecal *E. coli*, that human activity in the near vicinity increased the prevalence of MIs in these bacteria (Skurnik et al., 2006). Concerning the role of the antibiotic selective pressure, no published studies have demonstrated the direct *in vivo* selection of resistance through the acquisition of an integron. One study has demonstrated the *in vivo* selection of resistance through a rearrangement of the GCs array within a class 1 MI under antibiotic selective pressure (Hocquet et al., 2011).

Recent *in vitro* studies have shown that antibiotics are able to induce integrase transcription, both in CIs and MIs, *via* the SOS response. The SOS response is a global regulatory network controlled by the transcriptional repressor LexA and induced by stress leading to direct or indirect DNA damage, such as damage resulting from exposure to some widely used antibiotics (fluoroquinolones, beta-lactams, trimethoprim, aminoglycosides; Guerin et al., 2009; Baharoglu et al., 2010; Cambray et al., 2011). The activation of the SOS response in bacteria results in integrase overexpression, which leads to the raise of GCs recombination events.

Clinical, veterinary, and environmental surveys have shown that bacteria harboring MIs are often associated with multidrug-resistant (MDR) phenotypes (Bass et al., 1999; Leverstein-van Hall et al., 2003; Biyela et al., 2004; Nijssen et al., 2005; Laroche et al., 2009). However, the MDR profile could not be linked only to the antibiotic-resistance GCs carried by the MIs, but also to other resistance genes located on MIs-containing plasmids and transposons. This way, MIs could be co-selected with the plasmid- and/or transposon-associated antibiotic-resistance genes (Laroche et al., 2009; Li et al., 2010). For example, co-selection of class 1 MIs on plasmids harboring a *tet* gene (involved in the tetracycline resistance) in oxytetracycline-contaminated environments has been reported (Li et al., 2010).

The link between MIs and antibiotic resistance is still controversial since several studies present divergent conclusions (Hoyle et al., 2006; Smith et al., 2007). Furthermore many data have to be interpreted with caution. Indeed, biases in the study of links between MIs and antibiotic resistance could be generated by the selective choice of antibiotic-specific resistant strains, leading to misinterpretation. Finally, this relationship between MIs and antibiotic resistance has mainly been studied in bacteria of clinical or veterinary interest, such as those within the family Enterobacteriaceae.

Otherwise, the environment contains a wide range of bacterial species and cultivation methods only permit the isolation of a small fraction (around 1%; Amann et al., 1995). Techniques based on the study of the metagenome have thus been developed to avoid this limitation. The combination of culturing and metagenomics approaches on environmental ecosystems has highlighted the roles of MIs in antibiotic-resistance dissemination. **Tables 1** and **2** present an extensive list of the studies that have quantified the occurrence of MIs in the environment, using either cultivation methods (**Table 1**) or cultivation independent methods (**Table 2**). Genetic methods presented in this review quantify the abundance of integrase genes in the total DNA from different ecosystems. In order to normalize the quantity of gene to the total bacterial communities, most authors have used quantification of the ubiquitous bacterial 16S rRNA encoding genes. By dividing the abundance of integrase genes by the number of 16S rRNA genes, authors were able to demonstrate relative abundance. This ratio corresponds to the integrase genes proportion in the total bacterial communities. However, some authors have multiplying the ratio by the average number of copies of the 16S rRNA encoding genes per bacteria; which is approximately four (Klappenbach et al., 2001), and other authors present their results as percentages. In order to integrate all relative abundance data from diverse studies, results have been normalized to the same ratio for the purpose of this review and the relative abundance corresponds to the percentage of MIs per bacterial cell (**Table 2**).

MIs IN THE ENVIRONMENT

There is growing evidence that the environment plays a role in the spread of antibiotic resistance among pathogenic strains. Many questions have been raised concerning the impact of the release of antibiotics and antibiotic-resistant bacteria on the environment or on human and animal health (Aminov, 2010). The distribution of MIs, and especially the class 1 MIs, in the environment is a growing focus of attention, as illustrated by the recent publications presented in **Tables 1** and **2**.

MIs have been described in a wide range of natural ecosystems, both aquatic (e.g., lakes, rivers, estuaries) and terrestrial. However, their distribution has been investigated mainly in human-impacted environments such as amended soils and aquatic ecosystems influenced by urbanization, agriculture, aquaculture, industrial waste, and even in indoor and outdoor dust.

MI OCCURRENCE IN "NATURAL" ENVIRONMENTS

Different authors have investigated the occurrence of class 1 MIs in ecosystems considered to be untouched or barely affected by anthropogenic influence, these are often termed "reference sites" and correspond in **Tables 1** and **2** to the "clean area."

Only a few teams have studied MIs abundance in soils. Gaze et al. (2011) reported a class 1 MIs relative abundance of 0.00576% (**Table 2**) by a metagenomic approach in soils with no history of organic amendment, whereas the same authors previously found no class 1 MIs in the bacterial culturable fraction, which was composed of Bacillaceae, Paenibacillaceae, and Pseudomonadaceae (Gaze et al., 2005). In a study on forest soils, 11 out of 24 isolated Enterobacteriaceae strains (45%) were found to contain class 1 MIs, but these MIs harbored no GCs (Srinivasan et al., 2008).

In aquatics environments, Wright et al. (2008) and Hardwick et al. (2008) found, using metagenomics approaches, a relative class 1 MIs abundance recovery from 0.02 to 4%, in estuarine and stream water/sediments/biofilms, and 2.65% in creek sediments (**Table 2**). Using cultivation-dependent methods, class 1 MIs were found in lake sediments, with a prevalence of 1–4% (Stokes et al., 2006; Gillings et al., 2008a). Some studies investigated the GCs content of class 1 MIs. More often, one to three GCs were present, mainly encoding unknown function. GCs implied in the resistance to QACs (*qac* alleles) were also frequently described and

Table 1 | Bacterial prevalence of class 1 and 2 MIs in different ecosystems (results from cultivation-dependent studies).

	Ecosystem	Sample	Class 1 MIs% (*n*)	Class 2 MIs% (*n*)	Taxonomic affiliation	Reference
Clean area	Lake	Sediment	2.1 ($n=192$)	–	NS	Stokes et al. (2006)
			1–3 ($n=192$)	–		Gillings et al. (2008a)
	Soil/lake	Sediment	2–4 ($n=200$)	–	NS	Stokes et al. (2006)
	Soil	Forest soil	45.8 ($n=24$)	–	Enterobacteriaceae	Srinivasan et al. (2008)
		Agricultural land	0 ($n=262$)	–	NS and QACs^{R}	Gaze et al. (2005)
	Karst	Drinking water source	0 ($n=436$)		*E. coli*	Laroche et al. (2010)
Anthropogenic impacted***	River	US from the WWTP	0 ($n=75$) [a]	–	NS	Li et al. (2009)
			3 ($n=65$) [b]	–		Li et al. (2010)
			4.4 ($n=45$)	2.2 ($n=45$)	*E. coli*	Oberlé et al. (2012)
			6 ($n=301$) [c]			Koczura et al. (2012)
		DS from the WWTP	8 ($n=50$)	0 ($n=50$)		Oberlé et al. (2012)
			14 ($n=322$) [c]			Koczura et al. (2012)
			9.1 ($n=163$) [a]	–	NS	Li et al. (2009)
			86.2 ($n=87$) [b]	–		Li et al. (2010)
		Water	17.1 ($n=117$)	4.3 ($n=117$)	*E. coli***	Figueira et al. (2011)
			41 ($n=500$)	–	*E. coli*	Chen et al. (2011)
			58.1 ($n=43$)	–	MDR Enterobacteriaceae	Biyela et al. (2004)
			7.6 ($n=183$)	2.7 ($n=183$)	Enterobacteriaceae	Ozgumus et al. (2009)
			23 ($n=87$)	–	Enterobacteriaceae ^{R} and *Aeromonas* spp.^{R}	Guo et al. (2011)
			27.7 ($n=65$)	–		
		Water/sediment	13 ($n=32$)	3.1 ($n=32$)	MDR *E. coli*	Roe et al. (2003)
			44 ($n=313$)	–	*Aeromonas* sp.	Schmidt et al. (2001)
	Lake	Water	21 ($n=14$)	0	MDR *E. coli*	Dolejská et al. (2009)
	Estuaries	Water	8.9 ($n=279$)	1.4 ($n=279$)	*E. coli*	Laroche et al. (2009)
			29.6 ($n=54$)	7.4 ($n=54$)	amp^{R} Enterobacteriaceae**	Henriques et al. (2006)
			21 ($n=57$)	–	amp^{R} *Aeromonas* sp.**	
			3.6 ($n=3000$)	–	colif., *Pseudo.* And *Vibrio.**	Rosser and Young (1999)
	Hospital	wastewater	54.9 ($n=302$)	–	Enterobacteriaceae ^{R} and *Aeromonas* spp.^{R}	Guo et al. (2011)
			48.4 ($n=184$)			
			6 ($n=50$)	0 ($n=50$)	*E. coli*	Oberlé et al. (2012)
	Retirement home	wastewater	36 ($n=50$)	0 ($n=50$)	*E. coli*	Oberlé et al. (2012)
	WWTP	Raw effluent	15.1 ($n=643$) [c]		*E. coli*	Koczura et al. (2012)
		Treated effluent	11.5 ($n=174$) [c]			
		Activated sludge	3.7 ($n=378$) [c]			
		Raw effluent	10 ($n=61$)	8 ($n=61$)	Enterobacteriaceae and *Aeromonas* spp.**	Moura et al. (2007)
		Treated effluent	40 ($n=94$)	2 ($n=94$)		
		Activated sludge	61 ($n=35$)	6 ($n=35$)		
		Raw effluent	7.4 ($n=95$)	0 ($n=95$)	Enterobacteriaceae and *Aeromonas* spp.**	Moura et al. (2012)
		Treated effluent	4.6 ($n=131$)	0 ($n=131$)		
		Activated sludge	≈3 ($n=169$)	0.6 ($n=169$)		

(Continued)

Table 1 | Continued

	Ecosystem	Sample	Class 1 MIs% (*n*)	Class 2 MIs% (*n*)	Taxonomic affiliation	Reference
		Raw effluent	20.4 ($n=54$)	–	LF Enterobacteriaceae and *Aeromonas* spp.**	Ma et al. (2011a)
		Treated effluent	38.9 ($n=54$)	–		
		Activated sludge	30.9 ($n=81$)	–		
		Raw effluent	10	–	*E. coli***	Ferreira da Silva et al. (2007)
		Treated effluent	9.6	–		
		Raw effluent	19.1 ($n=204$)	4.9 ($n=204$)	*E. coli***	Figueira et al. (2011)
		Treated effluent	22.3 ($n=117$)	4.3 ($n=117$)		
		Raw effluent	16.4 ($n=49$)	0 ($n=49$)	*E. coli*	Oberlé et al. (2012)
		Treated effluent	8.5 ($n=49$)	2 ($n=49$)		
		Treated effluent[a]	14 ($n=179$)	–	NS	Li et al. (2009)
		Treated effluent[b]	97.4 ($n=189$)	–		Li et al. (2010)
		Activated sludge	33 ($n=109$)	–	LF Enterobacteriaceae**	Zhang et al. (2009b)
		Activated sludge	1 ($n=193$)	–	*E. coli* sul^R	Díaz-Mejía et al. (2008)
	Reed bed	Sediment	14.9 ($n=127$)	–	NS and $QACs^R$	Gaze et al. (2005)
	GWTP	AC biofilm	30 ($n=192$)	–	NS	Gillings et al. (2008a)
	Soil		6.6 ($n=500$)	10.2 ($n=500$)	NS + $antibiotic^R$	Byrne-Bailey et al. (2010)
			6.6 ($n=213$)	–	tet^R strains	Agerso and Sandvang (2005)
		Manured soil	89.3 ($n=56$)	–	Enterobacteriaceae	Srinivasan et al. (2008)
	Soil/pig slurry		6.2 ($n=531$)	9.6 ($n=531$)	NS + $antibiotic^R$	Byrne-Bailey et al. (2009)
	Compost		7.6 ($n=136$)	–	*E. coli* **	Heringa et al. (2010)
	Urban dust	Indoor	≈2 ($n=183$)	–	*E. coli* sul^R	Díaz-Mejía et al. (2008)
		outdoor	≈15 ($n=116$)	–		

*n, Number of isolated strains; LF, lactose fermenting; GWTP, ground water treatment plant; AC, activated carbon; NS, non-selective; US and DS, upstream (US) or downstream (DS) from the WWTP discharge in the receiving river; MDR, multidrug resistant; *coliform, Pseudomonas-like and Vibrio-like; **the taxonomic affiliation is based on 16S rRNA gene sequencing; ***impacted environment by urban and/or agricultural activities (sewage/industrial/WWTP/animal husbandaries facilities/fishponds/organic amendment); [a] the WWTP specifically treated effluents from a penicillin production facilities; [b] the WWTP specifically treated effluent from an oxytetracycline production facilities; [c] prevalence comprise both class 1 and 2 MIs; $QACs^R$, quaternary ammonium compounds resistant strains; Enterobacteriaceae and Aeromonas spp.^{R} refer to selected strains resistant to at least one antibiotic; amp^R, ampicillin resistant, sul^R, sulfonamide resistant, tet^R, tetracycline resistant; "≈": values have been extracted from graph.*

antibiotic-resistance GCs were rarely found (Gillings et al., 2008c, 2009a).

ENVIRONMENTAL SOURCE OF MIs

The class 1 MIs are ubiquitous elements naturally occurring in the environment, and different studies suggest that these elements emerged from ancestral environmental CIs (Rowe-Magnus et al., 2001; Mazel, 2006). Following the discovery of several class 1 MIs lacking resistance genes in environmental samples and located on the bacterial chromosome (Stokes et al., 2006; Gillings et al., 2008a), an evolutionary model was proposed and is now well documented (Gillings et al., 2008a; Labbate et al., 2009; Cambray et al., 2010; Stokes and Gillings, 2011). This model involves a succession of evolutionary recombination events, which facilitated the spread of class 1 MIs among pathogenic bacteria. These events led to the association of an "ancient" chromosomal class 1 MI with mobile functions of a *Tn*402-like transposon, and the acquisition of a *qacE* and *sul1* genes. During this evolution, deletions, insertions, and other rearrangements finally shaped the 3′CS of current class 1 MIs found in clinical isolates, as well as their inclusion in larger mobile platforms (plasmids and transposons), resulting in the spread of these elements among a broad range of bacteria, including pathogenic species (Gillings et al., 2008a; Labbate et al., 2009). Finally, it has been suggested that the class 1 MIs were probably widely distributed in Proteobacteria before the antibiotic era (Stokes and Gillings, 2011). These authors suggested that these class 1 MIs were unlikely to have GCs encoding antibiotic resistant determinants, and that they further evolved by acquisition of the 3′CS and antibiotic-resistance GCs. Nevertheless, a class 1 MI found in a *Pseudomonas* isolate recently recovered from 15,000- to 40,000-years-old Siberian permafrost with all the characteristics of a typical clinical class 1 MI, i.e., 5′CS and 3′CS, an antibiotic resistant GC (*aadA2* encoding resistance determinants to streptomycin and spectinomycin), localization on a mobile element (Tn5045 transposon), contradicts this hypothesis (Petrova et al., 2011).

ANTHROPOGENIC IMPACT ON MIs DISTRIBUTION

Rivers, seas, and lakes

Water is the main vector of pollutants in the environment and thus has received most attention. Furthermore, water bodies have been underlined as ideal vectors for the antibiotic-resistance

Table 2 | Concentration and relative abundance of class 1 MIs in total community DNA from different ecosystems.

		Samples	Characteristics	Class 1 MIs (*intI1*· L^{-1} or g^{-1})	Class 1 MIs relative abundance given by authors	Relative abundance recalculated (%)*	Reference
Clean area [a]	River/lake	Waters		$\approx 10^4$	≈0.01 [c]	4	Wright et al. (2008)
		Sediments		$\approx 10^3$–10^{4**}	≈0.001 [c]	0.4	
		Biofilm		$\approx 10^1$–10^{3**}	≈0.0001 [c]	0.04	
		Sediments		–	2.65 (max = 8) [d]	2.65 (max = 8)	Hardwick et al. (2008)
		Creek, GWTP, and pond biofilms		–	4.5 (min = 1/max = 9) [d]	4.5 (min = 1/max = 9)	Gillings et al. (2008c)
		River water		$< 10^6$	<0.0001 [c]	< 0.04	Lapara et al. (2011)
	Estuary	Waters		$\approx 10^3$–10^4	≈0.00005–0.0001 [c]	0.02–0.04	Wright et al. (2008)
		Sediments		$\approx 10^{4**}$	≈0.00005 [c]	0.02	
	Soil			–	0.0036 [e]	0.00576	Gaze et al. (2011)
Anthropogenic-impacted area [b]	River/lake	Waters	Industrial polluted	$\approx 10^3$–10^4	≈0.01 or 0.05 [c]	2 and 4	Wright et al. (2008)
		Sediments	Industrial polluted	$\approx 10^3$–10^{4**}	≈0.1 or 0.005 [c]	2 and 40	
		Biofilm	Industrial polluted	$\approx 10^2$–10^{4**}	≈0.0005 [c]	0.2	
		Waters microcosms	Initial (day 0)	$\approx 10^4$	≈0.0001 [c]	0.04	
		incubated at 23°C	Cadmium, 0.1 mM	$\approx 10^4$–10^5	≈0.001 [c]	0.4	
		during 7 days	Tetracycline, 30 mg L^{-1}	$\approx 10^3$–10^5	≈0.01 [c]	4	
			Control	$\approx 10^2$–10^3	≈0.0001 [c]	0.04	
		Sediments	Industrial area	–	≈1.5 (max = 4.294 for	6 (max = 17)	Rosewarne et al. (2010)
			residential area		the sewage input) [f]		
			DS a sewage output				
			Agricultural/clean area	–	≈0.1 (mini = 0.02) [f]	0.4	
		Waters	Urban and agricultural influenced	$\approx 10^7$–10^8	≈0.000005–0.005 [c]	0.002–2	Luo et al. (2010)
		Sediments	Urban and agricultural influenced	$\approx 10^{11**}$			
		River Waters	Urban and industrial polluted	$\approx 10^5$	between 10^2 and 10^3 [g]	–	Zhang et al. (2009a)
		Lake Waters	Urban and industrial polluted	$\approx 10^6$	between 10^3 and 10^4 [g]	–	
		Lake Sediments	Urban and industrial polluted	$\approx 10^{11}$	between 10^3 and 10^4 [g]	–	
		River Water	DS of a sewage output	≈ 1–6×10^6	≈0.0005–0.005[c]	0.2–2	Lapara et al. (2011)
		River sediment	DS of a sewage output	2.4–$2.5 \times 10^{6**}$	–	0.05	
		Lake water	Far DS of a sewage output	ND	ND	ND	
		Lake sediment		4.9–$7.7 \times 10^{5**}$	–	0.02	
	Estuary	Waters	Industrial polluted	$\approx 10^4$	≈0.001 [c]	0.4	Wright et al. (2008)

	Sediments	Industrial polluted	$\approx 10^{4**}$	≈ 0.0001 [c]	0.04	
WWTP	Raw effluent	CAS	2.04×10^{10}	1.46×10^{5} [g]	–	Zhang et al. (2009a)
	Treated effluent	CAS	1.20×10^{9}	1.48×10^{5} [g]	–	
	Activated sludge	CAS	2.49×10^{12}	1.17×10^{5} [g]	–	
	Raw effluent	CAS	$\approx 10^{11}$ and 10^{12}	$\approx 10^{6}$ [g]	–	Zhang et al. (2009b)
	Treated effluent	CAS	$\approx 10^{9}$	$\approx 10^{5}$ and 10^{6} [g]	–	
			$\approx 10^{7}$	$\approx 10^{4}$ [g]	–	
		After disinfection step	$\approx 10^{6}$	$\approx 10^{3}$ [g]	–	
	Activated sludge		$\approx 10^{9}$	$\approx 10^{2}$–10^{5} [g]	–	
	Digested sludge		$\approx 10^{8}$ and 10^{11}	$\approx 10^{1}$ and 10^{2} [g]	–	
	Treated effluent		$\approx 1.8 \times 10^{7}$	0.009 [c]	3.6	Lapara et al. (2011)
	Sludges		$5.13 \times 10^{9**}$	≈ 0.01 [c]	4	Ma et al. (2011b)
			–	≈ 0.0004–0.0015 [c]	0.16–0.6	Ghosh et al. (2009)
			1.0 and 1.3×10^{12}	≈ 0.01–0.1 [c]	4–40	Diehl and Lapara (2010)
	Digested sludge	QACs + ATB polluted	–	1.01 [e]	1.616	Gaze et al. (2011)
	Treated sludges	limed and dewatered	–	0.56 [e]	0.896	
	Reed bed cores	QACs polluted	–	0.65 [e]	1.04	
GWTP	Biofilter	Raw influent	8.0 and 9.28×10^{4}	287.0 and 309.3 [g]	–	Zhang et al. (2009a)
		Treated effluent	1.29 and 1.39×10^{4}	194.9 and 177.4 [g]	–	
		Biofilms	ND	856.9 and 823 [g]	–	
Soil	Pig slurry	Initial	–	0.0002 [e]	0.00032	Byrne-Bailey et al. (2010)
	amendment	1 day PA	–	0.01 [e]	0.016	
	experiments	21 day PA	–	0.008 [e]	0.0128	
		90 day PA	–	0.003 [e]	0.0048	
		289 day PA	–	0.004 [e]	0.0064	
		1 month PA	–	0.36 [e]	0.576	Gaze et al. (2011)
		12 month PA	–	0.02 [e]	0.032	
		24 month PA	–	0.01 [e]	0.016	
Animal waste	Pig slurry	Antibiotic (tylosin)	–	0.21 [e]	0.336	
		treated pig	–	0.21 [e]	0.336	Byrne-Bailey et al. (2010)

**The relative abundance was calculated using the formula: [(intI/16S) × 4 × 100], with four being the average number of copies of the gene encoding 16S rRNA per bacterial cell, according to the ribosomal RNA database (Klappenbach et al., 2001). **The results are expressed as copies·g^{-1}.*

[a] Represent natural environments without hospital proximity, WWTP, agriculture or animal husbandries facilities or no historical organic amendment practice; [b] impacted environment by urban and/or agricultural activities (sewage/industrial/WWTP/animal husbandries facilities/fishponds/organic amendment); [c] the relative abundance of integron was calculated per 16S rRNA encoding gene (intI/16S); [d] the relative abundance of integron was calculated per percent of bacterial cells [(intI/16S) × 4 × 100]; [e] the relative abundance of integron was calculated per percent of bacterial cells [(intI/16S) × 2.5 × 100]; [f] the relative abundance of integron was calculated per percent of 16S rRNA encoding gene [(intI/16S) × 100]; [g] the relative abundance was calculated by dividing the number of gene per the amount of total extracted DNA; PA, post-application; ND, not detected; GWTP, ground water treatment plant; QACs, quaternary ammonium compounds; ATB, antibiotic; DS, downstream; CAS, conventional activated sludge system; "≈": values have been extracted from graphs.

dissemination (Lupo et al., 2012). Indeed, compared to the "natural" waters previously described, the prevalence of class 1 MIs-containing strains is higher in known polluted waters (**Table 1**). The variation of results observed among studies may depend on many factors, such as the selected bacterial species, the applied culture method (selective or not selective), as well as the sample characteristics (e.g., sediment or water, occurrence of rain events before sampling, close location of a wastewater discharged site). Using metagenomic approaches, urban and agricultural activities were positively associated with class 1 MIs. High concentrations of class 1 MIs were found in a Chinese river located in an urban and agriculturally influenced region, with around 10^7–10^8 copies $\cdot$ L^{-1} and 10^{11} copies $\cdot$ g^{-1} of sediment (Luo et al., 2010), whereas in a clean area, concentrations of class 1 MIs were found to be around 10^4 copies $\cdot$ L^{-1} and 10^3–10^4 copies $\cdot$ g^{-1} of sediments (Wright et al., 2008). Zhang et al. (2009b) found a significant enrichment of class 1 MIs into the Yangtze river along its course through the Nanjing city, highlighting the impact of urban areas on rivers. Also, the relative abundance of class 1 MIs has been strongly correlated with the contribution of treated sewage output flow in the receiving river sediment (Rosewarne et al., 2010). This has been confirmed in a recent study carried out by Lapara et al. (2011), underlining the role of the wastewater treatment plant (WWTP) in the dissemination of class 1 MIs in the environment. Otherwise, fish farming has been shown to significantly elevate the prevalence of class 1 MIs in motile Aeromonads in river waters. The MIs identified contained *dfr* GCs encoding trimethoprim determinants, and their occurrence correlated with the administration of combined sulfonamide/trimethoprim drugs in freshwater fish farms (Schmidt et al., 2001). In polluted estuaries, the prevalence of class 1 MIs appears to be less important than in the aquatic ecosystems previously described, with values ranging between 2.7 and 14.7% (Laroche et al., 2009). Nevertheless, it has been observed that in anthropogenically impacted estuaries the relative abundance was around 10 times more than in an unpolluted reference estuary (Wright et al., 2008; **Table 2**). The authors did not show any influence of the tide, the relative MIs abundance being similar during ebb or flood tides.

Studies involving effluents of factories which produce antibiotics showed that antibiotic production could have an effect on the prevalence of MI-containing bacteria in the receiving river (Li et al., 2009, 2010). In these two studies, the impact differed according to the industry production, although the effluent treatment processes were equivalent in the two industries (anaerobic digestion following by activated sludge process without disinfection step). Indeed, the penicillin production effluents elevated the prevalence of class 1 MIs-harboring strains in the river, from 0% upstream of the discharge to 9.1% after the treated effluent was discharged whereas the oxytetracycline production effluents elevated the MIs prevalence in the river from 3% upstream of the discharge to 86.2% downstream (Li et al., 2009, 2010). Moreover, the authors suggested that some *Pseudomonas* sp. and *Bacillus* sp. isolates harbored simultaneously up to seven different class 1 MIs per bacteria, from the effluent of the oxytetracycline factory, as well as in the receiving river. In comparison, the bacteria from upstream of the WWTP harbored only one class 1 MI. More recently in a metagenomics study, authors observed a 6.7-fold enrichment of class 1 MIs in river sediments downstream of a treated WWTP effluent discharge point from an antibiotic production complex (Kristiansson et al., 2011).

However, the impact of anthropogenic activities is not limited to antibiotic pressure alone, since similar observations have been made in environments without sources of antibiotics input. An Australian study has correlated the rise of the relative abundance of class 1 MIs with environmental parameters (Hardwick et al., 2008). When the environmental conditions were more stressful to the bacteria, the relative abundance of class 1 MIs was higher. Industrial activities (mainly resulting in heavy metal contamination) also have been shown specifically to contribute to the increase of class 1 MIs relative abundance (Wright et al., 2008; Rosewarne et al., 2010). It has been shown that adding tetracycline or cadmium to a water stream in microcosm experiments increased the MIs relative abundance by a factor of between 10- and 100-fold (Wright et al., 2008). The co-selection of resistance genes with heavy metal such as mercury resistance has been previously described (Aminov and Mackie, 2007). Class 1 MIs have been described on the *Tn*21 transposon which also contains a mercury resistance operon (Liebert et al., 1999). Antiseptic agents as QACs have also been shown to be associated with a higher prevalence of class 1 MIs (Gillings et al., 2008c). In QACs contaminated reed bed, it was shown that 95% of the isolated strains with class 1 MIs harbored a *qac* gene (Gaze et al., 2005). Heavy metals and QACs are thus probably involved in MIs dissemination and may have contributed to the MIs selection before the antibiotic era (Stokes and Gillings, 2011).

In anthropogenic-impacted waters, an important diversity of GCs has been recovered (Rosser and Young, 1999; Roe et al., 2003; Henriques et al., 2006; Taviani et al., 2008; Laroche et al., 2009; Li et al., 2009; Ozgumus et al., 2009; Verner-Jeffreys et al., 2009; Kumar et al., 2010; Rosewarne et al., 2010; Chen et al., 2011). Resistance to almost all families of antimicrobials has been recovered with various GCs: *aad*, *aac* (conferring resistance to aminoglycosides); bla_{CARB-2}, bla_{OXA}, bla_{P1} (conferring resistance to beta-lactams); *dfr* (conferring resistance to trimethoprim); *catB* (conferring resistance to chloramphenicol); *ereA* (conferring resistance to erythromycin); *arr* (conferring resistance to rifampicin); and *qac* (conferring resistance to QACs). Moreover, GCs with unknown function have been also commonly found. Several studies have characterized the total pool of integron GCs from environmental samples by using a PCR approach targeting only the GCs (*attC* sites) and not the integrase genes. They showed a huge GCs diversity mostly encoding unknown functions, and underlined the effect of both environmental and anthropogenic conditions on the GCs pool composition (Koenig et al., 2008, 2009, 2011; Huang et al., 2009; Elsaied et al., 2011). Anthropogenic activity thus increases the prevalence of class 1 MIs in microbial communities. These anthropogenic environmental changes result in an increase in transferable genetic elements potentially harboring resistance genes, and an ability to capture new resistance genes from autochthonous hosts. Antibiotic-resistance genes located in mobile genetic elements (plasmids, transposons, integrons) have been suggested to be "genetic pollutants" representative of human activities (Martinez, 2009a). Moreover, anthropogenic stresses has been suggested to facilitate the possible transfer of chromosomal

resistance genes to the mobile gene pool accelerating the evolution and the possible spread in human-pathogenic strains (Cattoir et al., 2008; Picão et al., 2008; Martinez, 2009a,b; Rahube and Yost, 2010).

Class 2 MIs are less prevalent than class 1 in polluted waters (0–7.4%), **Table 1**. In a culture-independent method survey, the low relative abundance rate of class 2 MIs from river has been underlined (Luo et al., 2010). These results suggest that their role in aquatic ecosystems is probably minor.

Sewage and wastewater treatment plants

Wastewater treatment plants are the interface between human waste and both the aquatic and soil environments (**Figure 2**). They collect effluents from diverse sources (such as hospital, private household, industries, animal husbandries), which contribute to the final ecosystem of the WWTP. These include the organics, chemicals, and microbiological wastes. Finally the WWTP ecosystem constitutes a "broth" where each element interacts with each other under a physical and chemical constraint resulting mainly in an organic degradation in the aqueous and solid phase. Microorganisms are key to the process resulting in organic and chemical degradation or transformation. The bacterial communities are organized in free biofilm entities (called bacterial flocs), which constitute the total biomass (the sludge). As suggested by many authors, the high bacterial density, due to the nutritional richness, indicates that WWTP are hot spots for horizontal gene transfer (Tennstedt et al., 2003). Moreover, the antibiotics potentially present in the WWTP could select antibiotic-resistant bacteria, as shown for erythromycin (Louvet et al., 2010), thereby enabling the persistence of antibiotic-resistance plasmids. It has been shown that sulfamethoxazole or amoxicillin at sub-inhibitory concentrations in activated sludge improved the stability of the pB10 plasmid in *E. coli* (Merlin et al., 2011). This co-existence of bacteria and antibiotics in WWTP increases the frequency of genetic variations (as recombination events) and the possible emergence of novel mechanisms of resistance (Baquero et al., 2008).

The highest concentrations of class 1 MIs ever described have been recovered from raw effluents with values comprised between

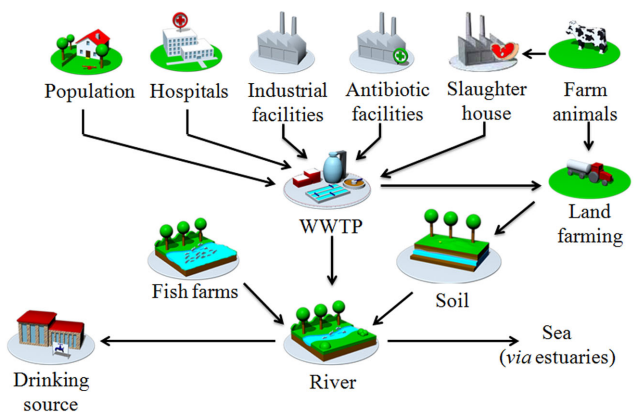

FIGURE 2 | Main route of MIs dissemination from anthropogenic sources to the environment (pictures derived from PILLS project: http://www.pills-project.eu).

10^{10} and 10^{12} copies $\cdot L^{-1}$ (**Table 2**). Class 1 MIs have been described at all stages of the WWTP process with variable prevalence or relative abundance (**Tables 1** and **2**), nevertheless their relative abundance in the final treated effluents highlights the inefficiency of the process to remove bacteria harboring these genetic elements (this will be described below in more details). In activated sludge, 3–61% of the isolated strains harbored a class 1 MI (**Table 1**). By metagenomics approach, the relative abundance of class 1 MIs varied (**Table 2**). These variations could be explained by the different methods of nucleic acids extraction as well as the primers used to detect the MIs. Nevertheless relative abundance up to 40% has been found suggesting that the activated sludge is a hot spot for class 1 MIs selection and/or dissemination. In addition, two studies showed that 12% of isolated plasmids from WWTP sludge carried MIs (Tennstedt et al., 2003; Moura et al., 2007), among which more than half were broad-host-range plasmids displaying very high transfer frequencies (Tennstedt et al., 2003).

As previously described in the aquatic ecosystems, the low prevalence of class 2 MIs in WWTP suggests that their role is probably minor. Although less than 10 publications have reported class 3 MIs, 2 of them have been described in *Delftia* sp. (*D. acidovorans* and *D. tsuruhatensis*) isolated from activated sludge (Xu et al., 2007). These class 3 MIs contained GCs of unknown function. Moreover, using molecular approach, class 3 MIs were detected in effluents from an urban WWTP and a slaughter house WWTP (Moura et al., 2010). These findings suggest that even if class 3 MIs play a minor role in clinical microbiology, their role in the environment is probably more extensive.

The analysis of GCs content from wastewater ecosystems showed a huge diversity of genes encoding antibiotic resistance: resistances to aminoglycosides with *aad*, *aacA* GCs; to beta-lactams with bla_{OXA}, bla_{VIM-2}, bla_{IMP}, bla_{P1}, bla_{GES-5}, and bla_{GES-7} GCs; to trimethoprim with *dfr* gene GCs; to chloramphenicol with *cat* and *cml* GCs; to erythromycin with *ereA* and *estX* GCs; to rifampicin with *arr* GCs; and to quinolones with *qnrVC4* GC (Tennstedt et al., 2003; Ferreira da Silva et al., 2007; Moura et al., 2007, 2012; Taviani et al., 2008; Li et al., 2009, 2010; Pellegrini et al., 2009, 2011; Zhang et al., 2009b; Xia et al., 2010; Girlich et al., 2011; Guo et al., 2011; Ma et al., 2011a; Scotta et al., 2011). A molecular approach describing the global pool of GCs in WWTP have shown a great diversity of GCs, mainly encoding for determinants implied in metabolic functions or unknown functions, suggesting the wide potential reservoir of GCs in WWTP (Moura et al., 2010).

Efficiency of WWTP process to remove MIs

While the WWTP reduced the bacterial load, it appears that the treatment is inefficient to remove both antibiotic resistant bacteria (Novo and Manaia, 2010; Luczkiewicz et al., 2010), and MIs-harboring bacteria.

As presented in **Tables 1** and **2**, the prevalence or relative abundance of MIs after the activated sludge process is not reduced, and is even often higher than in the raw effluent (Ferreira da Silva et al., 2007; Moura et al., 2007; Figueira et al., 2011; Ma et al., 2011a). These authors often concluded that activated process can remove bacteria, but do not reduce significantly the bacteria harboring class 1 MIs. When using abundance normalized to the total

DNA amount, same observation have been done (Zhang et al., 2009a), however in another study, these authors found that the effluent treatment process decreased the MIs rate (Zhang et al., 2009b). Nevertheless, normalization to DNA amount is critical as total community DNA usually contains DNA of non-bacterial origin. The removal of bacteria bearing antibiotic-resistance genetics elements by the WWTP is a new challenge for the future. Several studies have investigated the efficiency of different advanced processes such as UV treatment, membrane biological reactors, and chlorination, to remove bacteria carrying antibiotic-resistance genes (Auerbach et al., 2007; Garcia et al., 2007; Kim et al., 2010; Huang et al., 2011; Munir et al., 2011), but no studies have examined the effects on MIs. Recently, hospital effluents were shown to be potential sources of dissemination of MIs in the sewage network (Guo et al., 2011). Oberlé et al. (2012) noted a decrease of the prevalence of class 1 MIs in *E. coli* along the effluents treatment from the hospital to the WWTP and the receiving river continuum. As treatment of hospital effluents onsite is a growing and controversial question (Pauwels and Verstraete, 2006; Kümmerer, 2008; Ort et al., 2010; Escher et al., 2011), these data need confirmation by further studies in order to assess the impact of these specific effluents on the release of MIs in the WWTP.

Activated sludges are often used in agriculture as organic amendment (**Figure 2**). However, before their use, treatments in order to reduce their volume and improve stability are applied. Several studies have specifically investigated the potential of aerobic and anaerobic treatments to reduce class 1 MIs in activated sludges, demonstrating a better performance of anaerobic thermophilic process (50–60˚C) to decrease the relative abundance of class 1 MIs (Ghosh et al., 2009; Diehl and Lapara, 2010). However, dissimilar results have been obtained in same conditions by Ma et al. (2011b), suggesting that other factors may influence the MIs occurrence during the sludge digestion. Evidence of horizontal gene transfers in WWTP sludge has been shown by Merlin et al. (2011). They have shown that, horizontal transfer of pB10 plasmid occurred in sludge from the anaerobic digesters or from fixed biofilm reactors, with higher efficiency in fixed biofilm conditions.

Soil ecosystem and the animal wastes as sources of MIs

While the soil "resistome" is a vast original reservoir of resistance genes (D'Costa et al., 2006; Allen et al., 2010), manure has been shown to significantly increase the mobile genetic resistance elements pool (Heuer et al., 2011). Recent studies have highlighted the role of the amendment practice on the input of MIs in soil (Heuer and Smalla, 2007; Binh et al., 2009; Byrne-Bailey et al., 2010; Gaze et al., 2011), see **Table 2**. Moreover, studies on sewage sludge and pig slurry amendment showed that even if the prevalence of class 1 MIs decreased after the particular amendment (2 years and 10 months, respectively), the prevalence was still higher than in control soils without amendment (around 100 times more; Byrne-Bailey et al., 2010; Gaze et al., 2011). Some authors studied the GCs array of class 1 MIs introduced in soil *via* manure amendment and mainly found streptomycin and spectinomycin resistance *aadA* GCs (Heuer and Smalla, 2007; Binh et al., 2009). Class 2 MIs have been also identified from amended soils with relative high rates (Byrne-Bailey et al., 2009, 2010; Rodríguez-Minguela et al., 2009). The high antibiotics consumption in some animal husbandries, and their systemic application as food additives in the past, had probably significantly contributed to MIs dissemination in amended soils. Tschäpe (1994) showed that the streptothricin usage as food additive contributed to the dissemination of *sat* genes in amended soils *via* mobile genetic structures, such as the *Tn7* transposon carrying a class 2 MI usually bearing a streptothricin-resistant *sat2* GC.

Animal wastes (e.g., manure, poultry litter, and slurry) are the main vectors of MIs dissemination in soil. As recently reviewed by Heuer et al. (2011), only a few studies have investigated the reduction of some resistance genes following different processes, such as storage, composting, and anaerobic digestion (Chen et al., 2007, 2010; Heuer et al., 2008). Only composting was efficient in reducing the prevalence and absolute amount of erythromycin resistance genes (Chen et al., 2007). Concerning the MIs, one study reported that after 57 days of storage of manure at 20˚C, the class 1 MIs GCs array electrophoresis gel profiles were almost identical to that at the beginning of the experiment; however the GCs contents was not investigated (Heuer et al., 2008).

ROLE OF THE FOOD CHAIN

The food chain probably also takes place in the transit of MIs from the environments to the human. Indeed, bacteria harboring MIs have been recovered from a variety of aquatic living organisms, such as in prawns, with an *Enterobacter cloacae* harboring a class 1 MI (Gillings et al., 2009b); or in *Corbicula* with a class 1 MIs relative abundance of 4% (Wright et al., 2008); and in oysters where the uncommon class 3 MIs prevailed (Barkovskii et al., 2010). Transfers of MIs between animals and human occur and have been well reviewed by Stokes and Gillings (2011). Class 1 MIs have been also reported from biofilms of drinking water supplies (**Tables 1** and **2**; Gillings et al., 2008a; Zhang et al., 2009a). All these results underline the link, *via* the food chain, between the environmental MIs and the human or animal MIs.

CONCLUSION

As described in this review, MIs are efficient tools for bacterial adaptation and play a significant role in antibiotic resistance. Environmental studies demonstrated that anthropogenic impact lead to enrichment of class 1 MIs. More specifically, all factors leading to bacterial stress, such as antibiotics, QACs, or high concentrations of heavy metals resulted in the selection of class 1 MIs-harboring bacteria. Several hot spots of class 1 MIs dissemination have been identified, as agricultural manure amendment, WWTP, or industrial effluents. While these wastes are treated in varying degrees before their discharge, it appears that the current processes are inefficient to reduce MIs dissemination. This uncontrolled dissemination of MIs in the environment could represent a risk for human health.

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